

Figure 1

Screening Protocol for Haptotactic-Migration Inhibitors: V 3.0

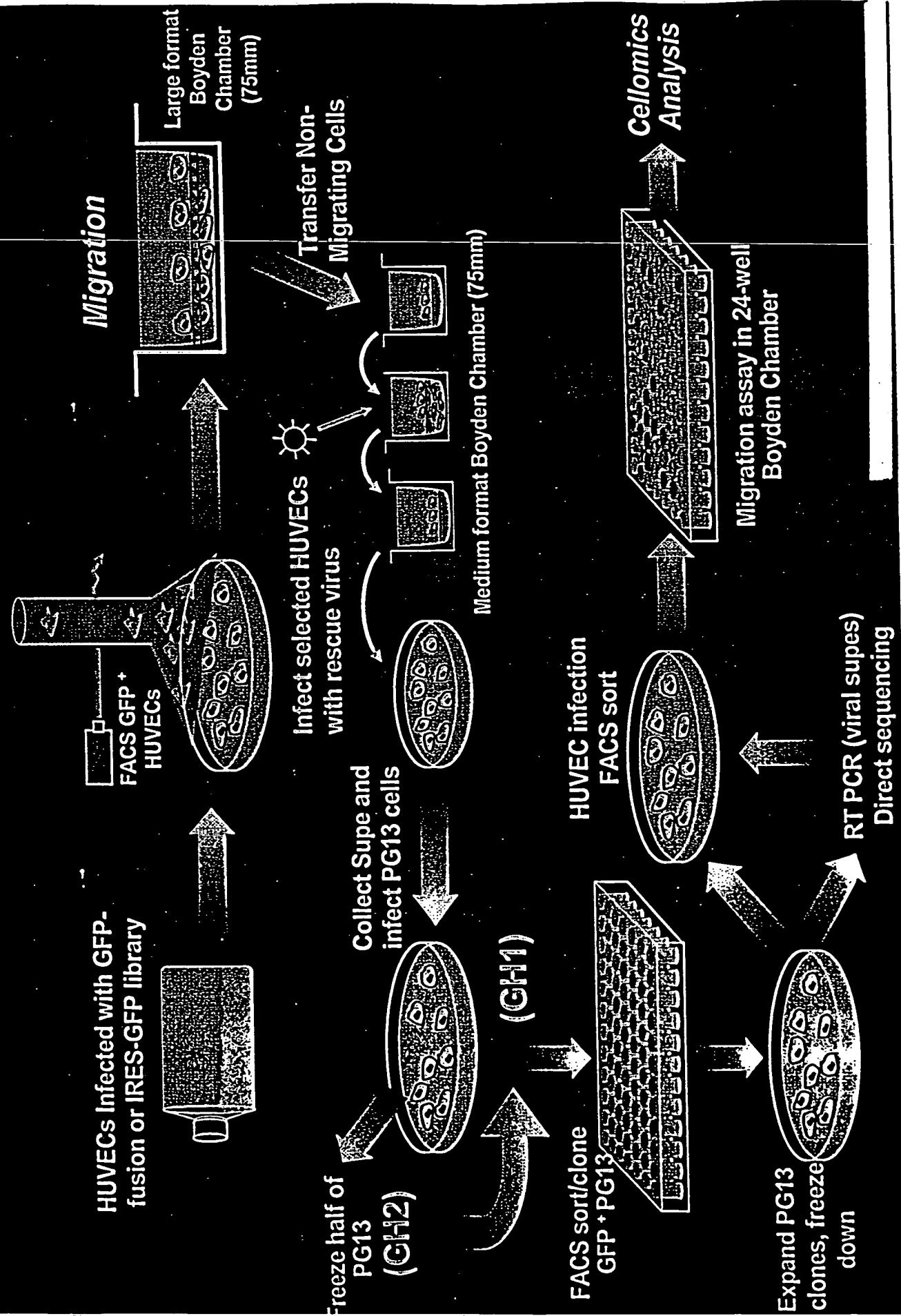
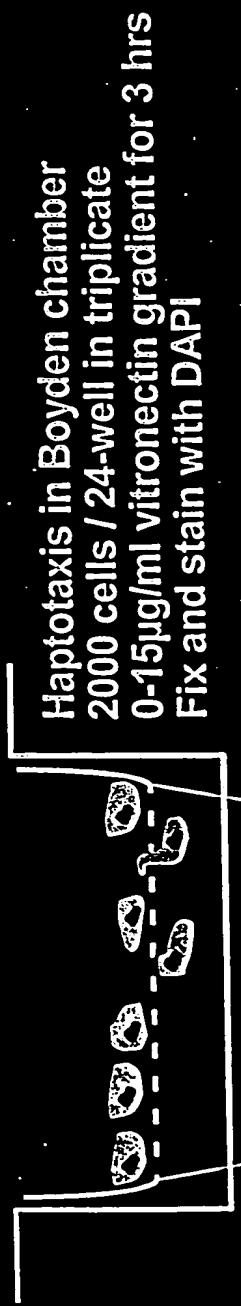


Figure 2

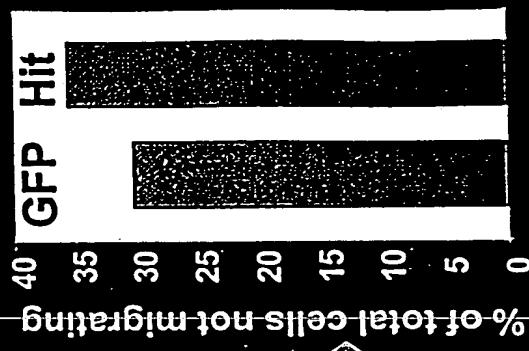
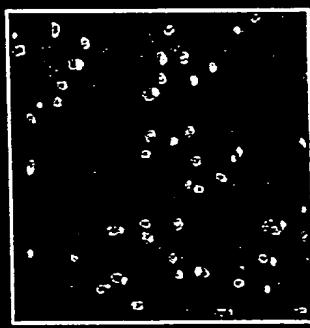
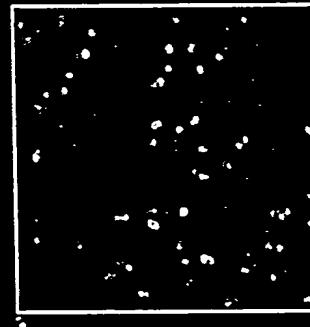
Cellomics Haptotaxis Assay



Haptotaxis in Boyden chamber
2000 cells / 24-well in triplicate
0-15 μ g/ml vitronectin gradient for 3 hrs
Fix and stain with DAPI

Count # cells on top / bottom
using Cellomics image processing

image 9 fields
well

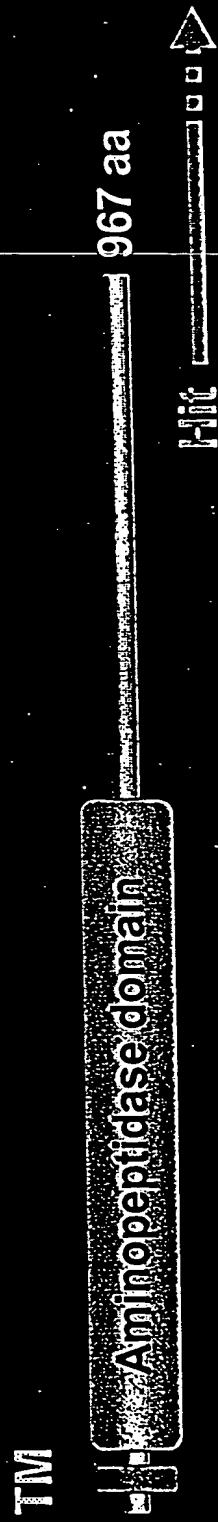


Data presented as
% cells NOT
migrating

- Cells directly imaged on membrane
- High accuracy in counting
- Distinguishes cells vs pores

Figure 3

GFP-CD13 / N-aminoopeptidase Screening Hit Inhibits Haptotaxis



- Type II membrane protein
- M1 metalloprotease
- Expressed in myeloid cells and angiogenic endothelial cells; upregulated by VEGF and bFGF in in HUVECS
- CD13 inhibitors impair tube formation, angiogenesis and tumor growth
- Expressed in RA synovia; T cell chemoattractant

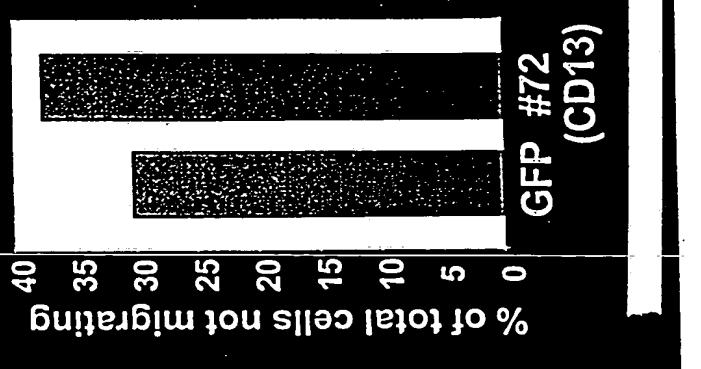
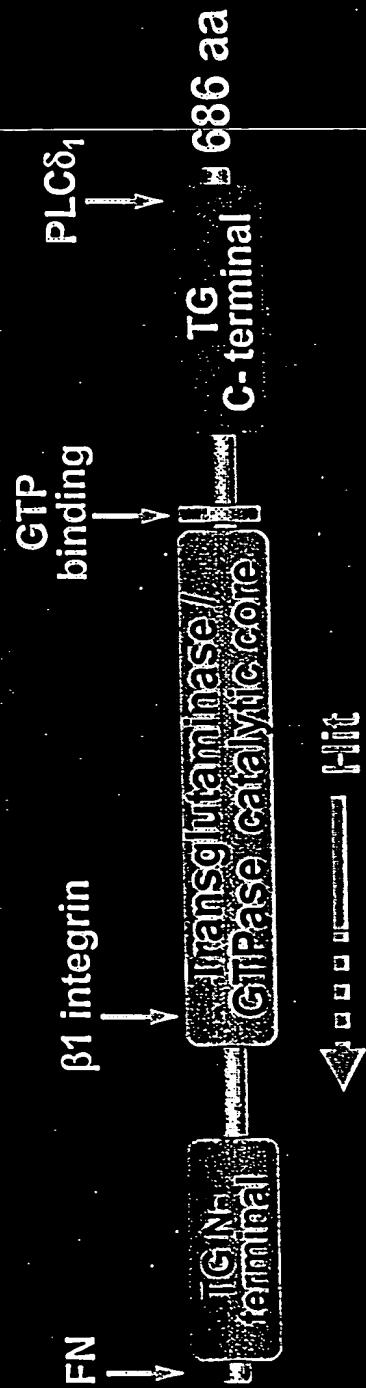


Figure 4

GFP-Transglutaminase II Screening Hit Inhibits Haptotaxis



- Antisense hit
- Multifunctional protein

- protein crosslinking
- (de)amidation (e.g Rho)
- GTPase mediates 7TM receptor signaling to PLC δ 1
- mediates integrin interactions
- Constitutively expressed at high levels in endothelial cells
- Expressed in endothelial cells during wound healing
- TG2 null mice exhibit impaired wound healing, autoimmunity and diabetes
- Recombinant transglutaminase promotes angiogenesis

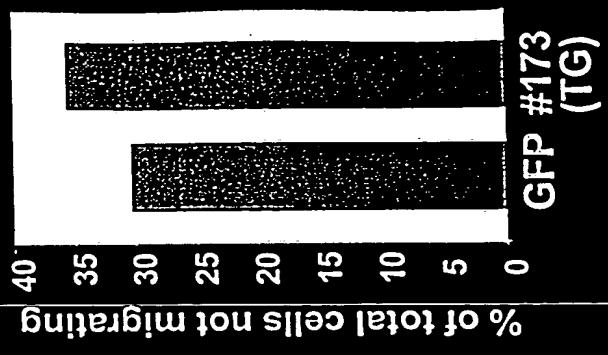
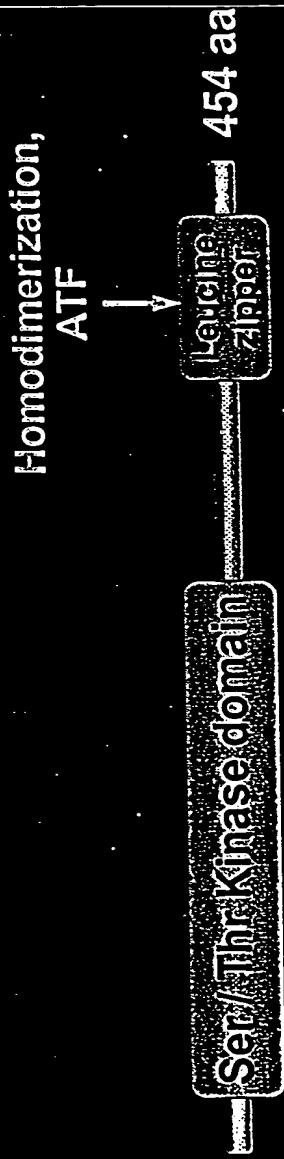


Figure 5

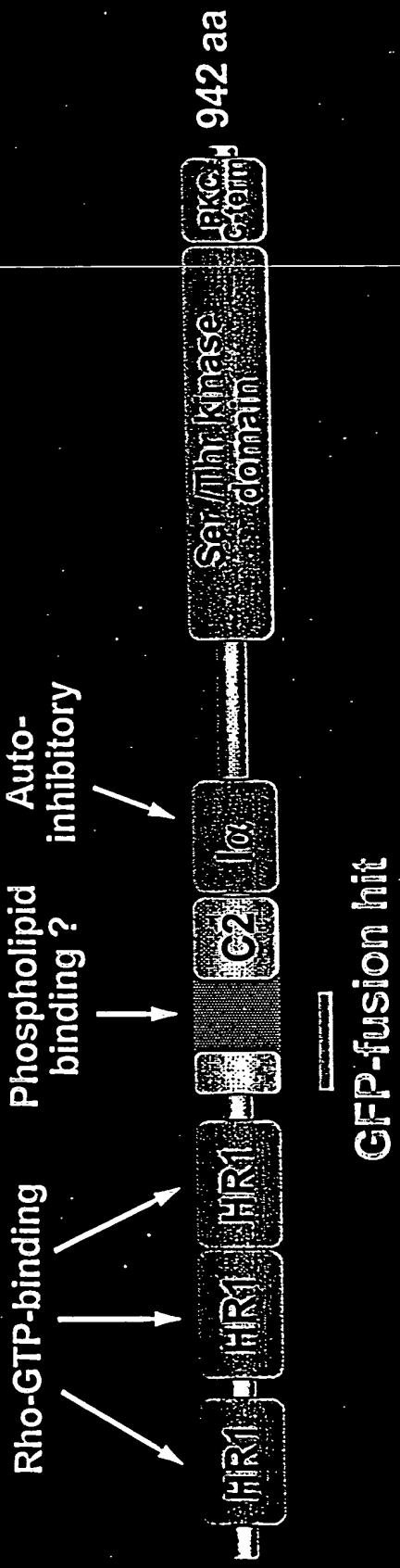
GFP-Zip Kinase Screening Hit Inhibits Haptotaxis



- Hit is 3' untranslated sequence
- Overexpression causes apoptosis in NIH 3T3 cells, recombinant protein stimulates contraction of smooth muscle
- Phosphorylates MLC₂₀ in vitro, activating myosin ATPase activity and mediates Ca²⁺-independent SMC contraction
- Localized to nucleus (NIH 3T3), myofibrils (SMC) and actin filaments?

Figure 6

GH1-54 Screening Hit Encodes a Fragment Of PRK1



GFP-fusion hit

- Cytoplasmic serine/threonine kinase related to PKC
- Hit GFP-fusion with part of C2 domain
- Binds Rho GTPase through N-terminus
- Regulated by activation loop phosphorylation by PDK1
- PRK activation by PDK1 is Rho-dependent and membrane-localized
- May regulate actin / myosin / microtubules



Figure 7

PRK1 mRNA Expression is Restricted to Endothelial Cells and PBMCs

Taqman expression profile of primary cells (polyA+ RNA)

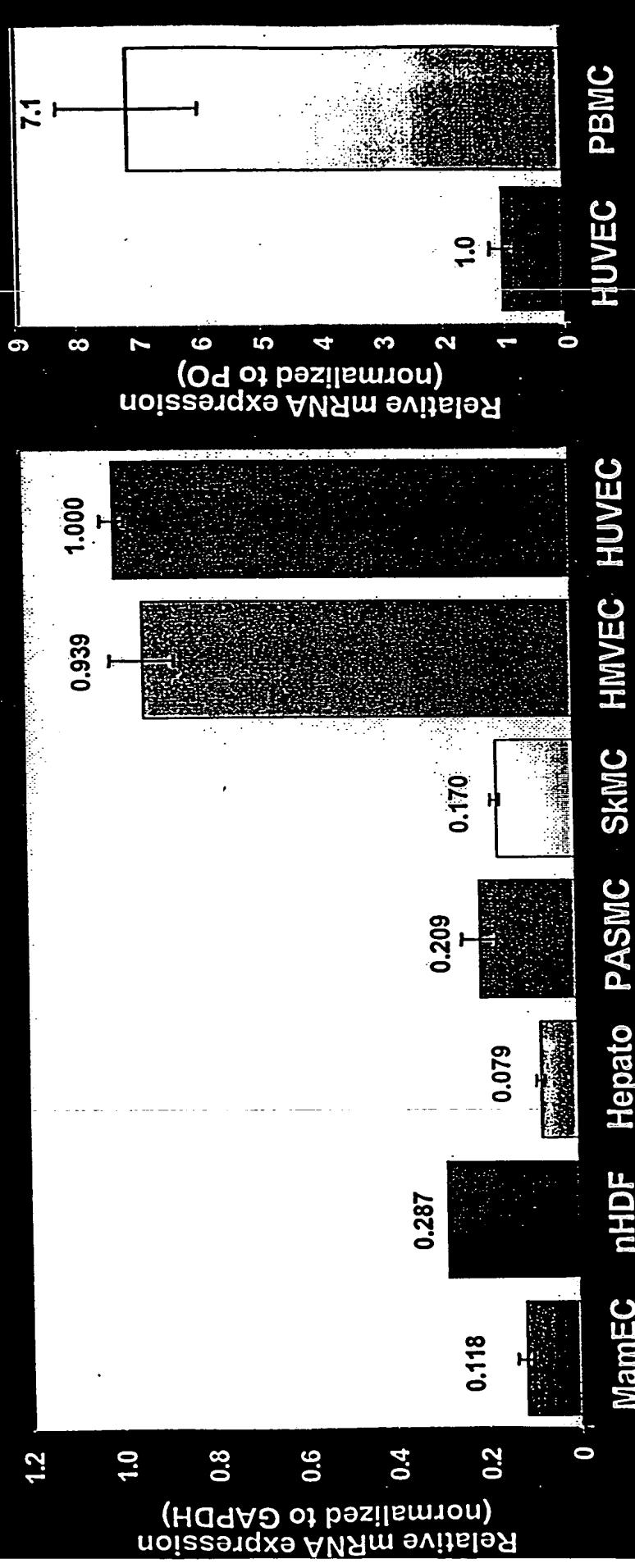


Figure 8

GFP-PRK-1 Screening Hit Inhibits Haptotaxis and Reduces $\alpha v \beta 3$ Levels

Haptotaxis on Vitronectin Gradient

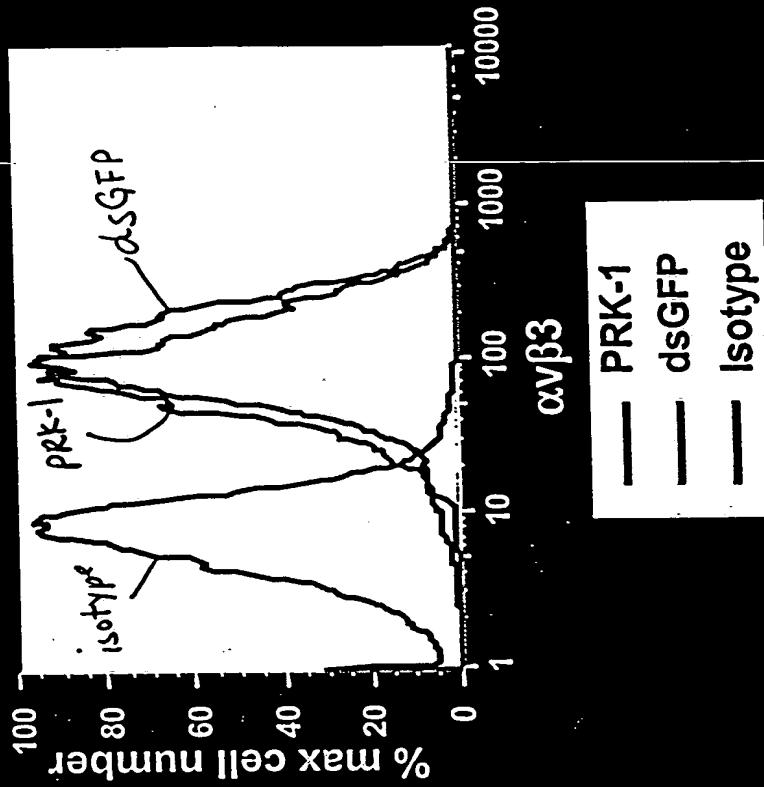
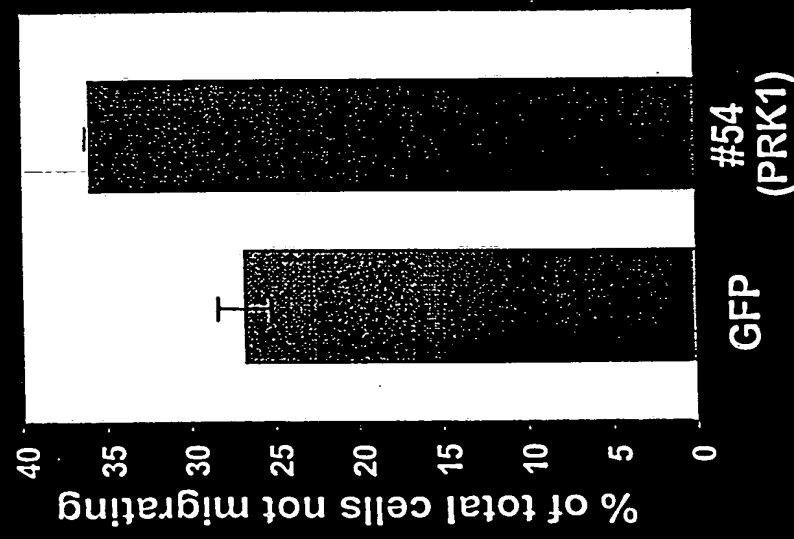


Figure 9

PRK-1 RNAi Reduces PRK-1 Message, Haptotaxis and $\alpha v \beta 3$ Expression

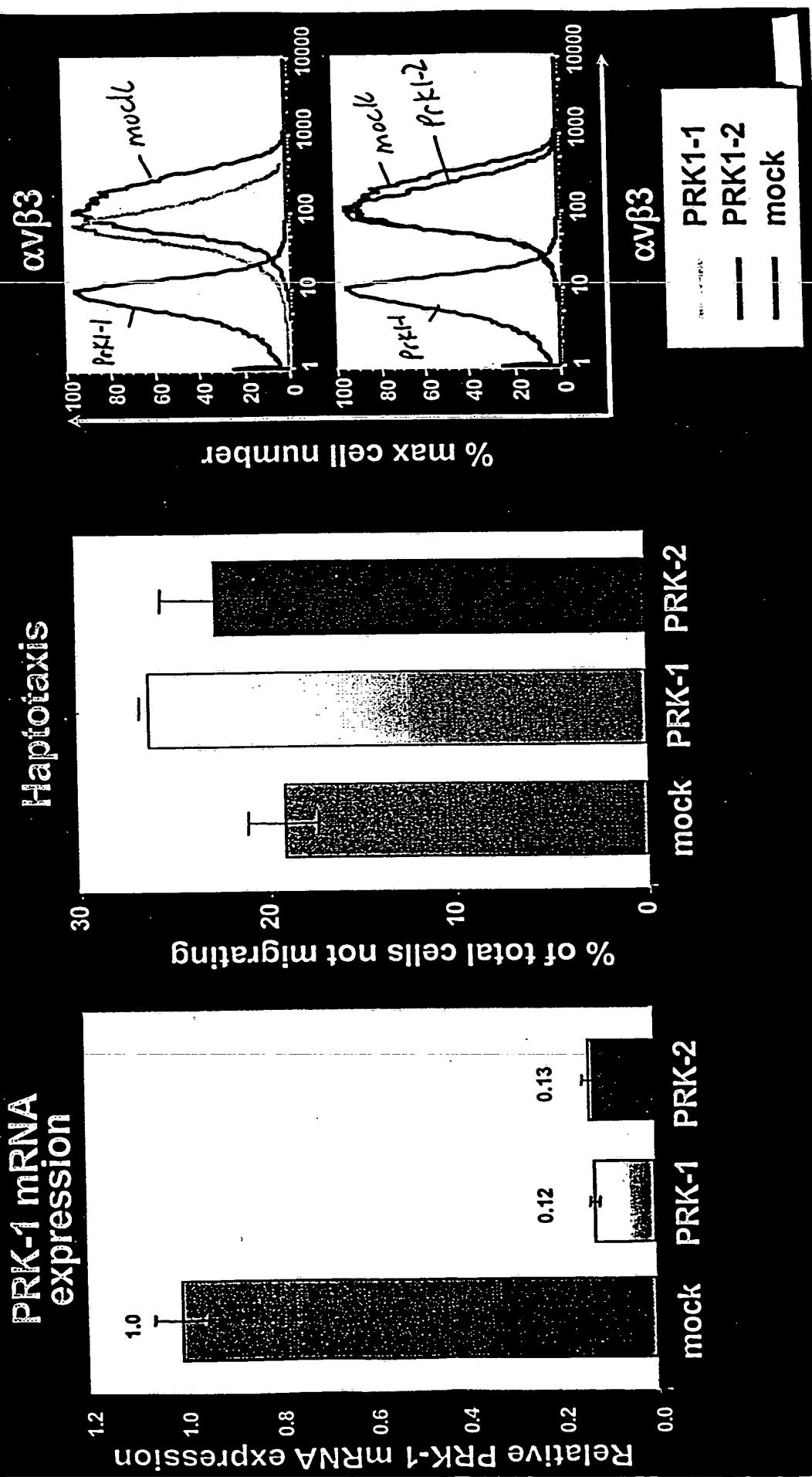
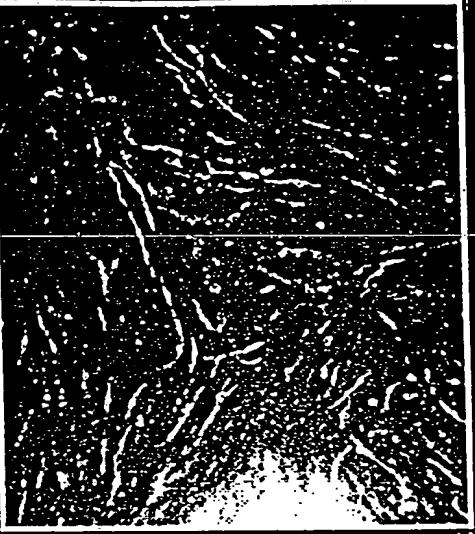


Figure 10

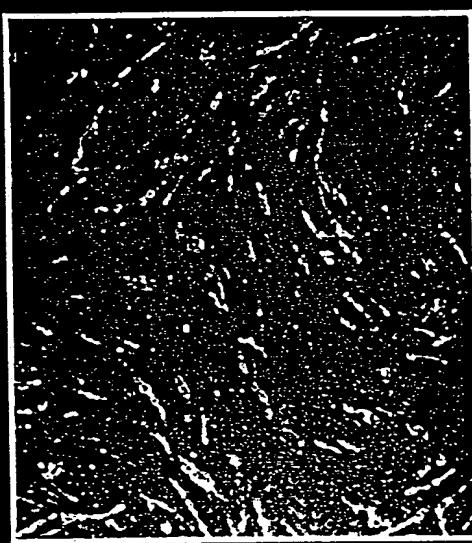
PRK-1 RNAi Reduces Tube Formation in the Co-culture Assay

5 day co-culture stained with anti-CD31-FITC

PRK1-2



PRK1-1



Mock

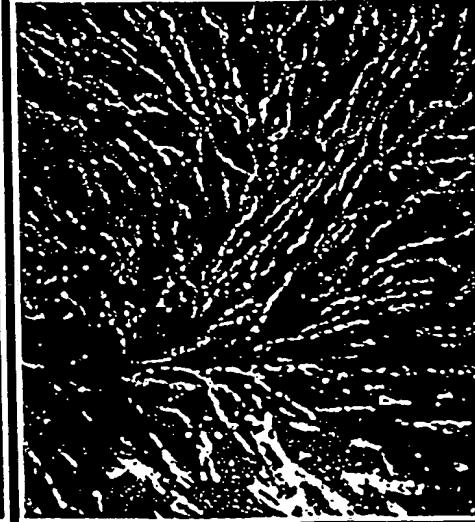
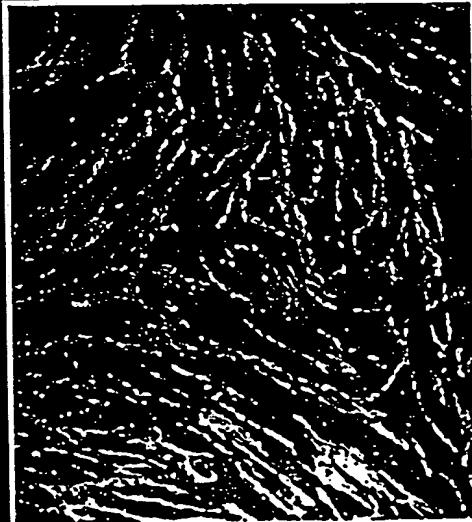


Figure 11

Ax1 and Gas6 were isolated in the Haptotaxis Screen

Ax1 Gas6 FNG3 FNG2 Tyrosine kinase domain
(Antisense hit)

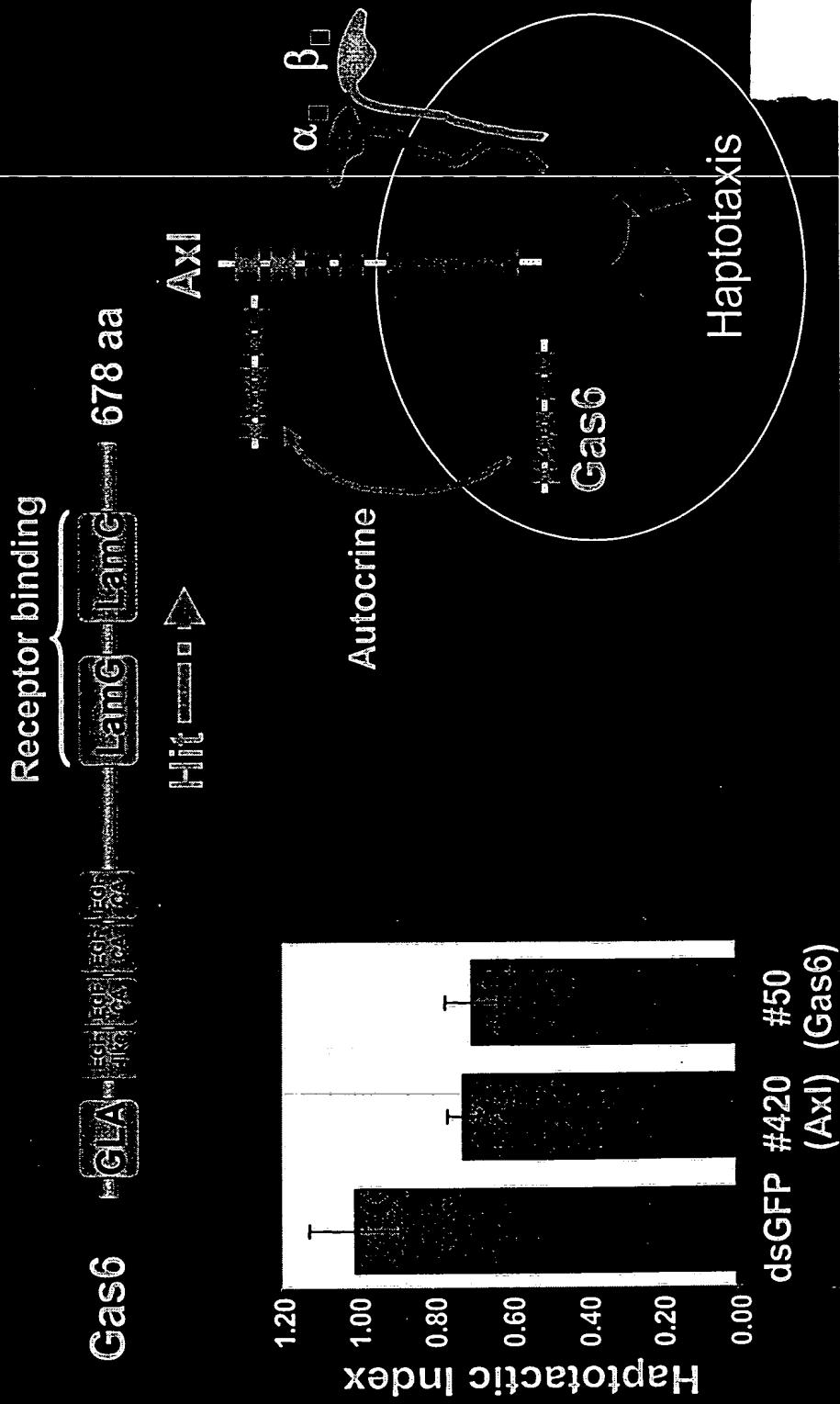
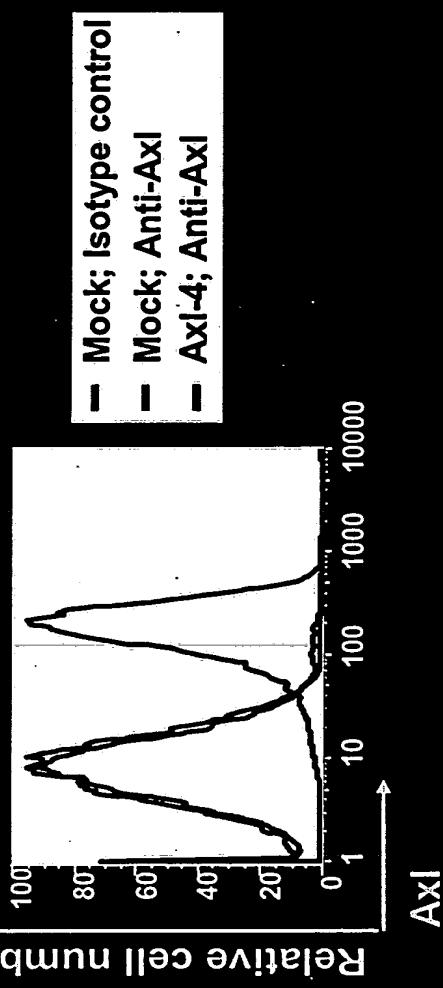


Figure 12

AxI-4 RNAi Knocks Down AxI Protein Expression and Phenocopies Screening Hit Inhibition of VN Haptotaxis FACS at 48 hrs post transfection



VN Haptotaxis at 48 hrs post transfection

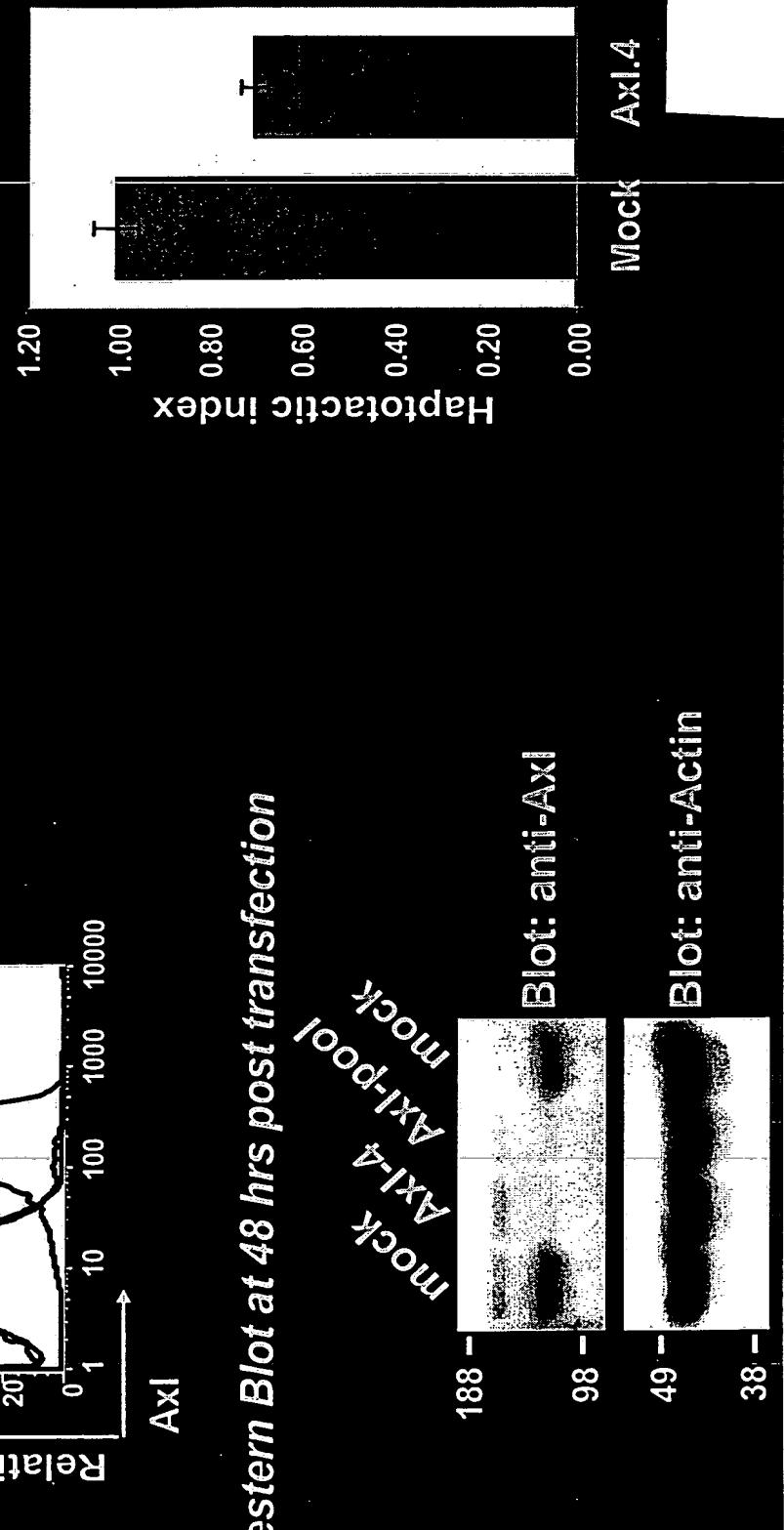
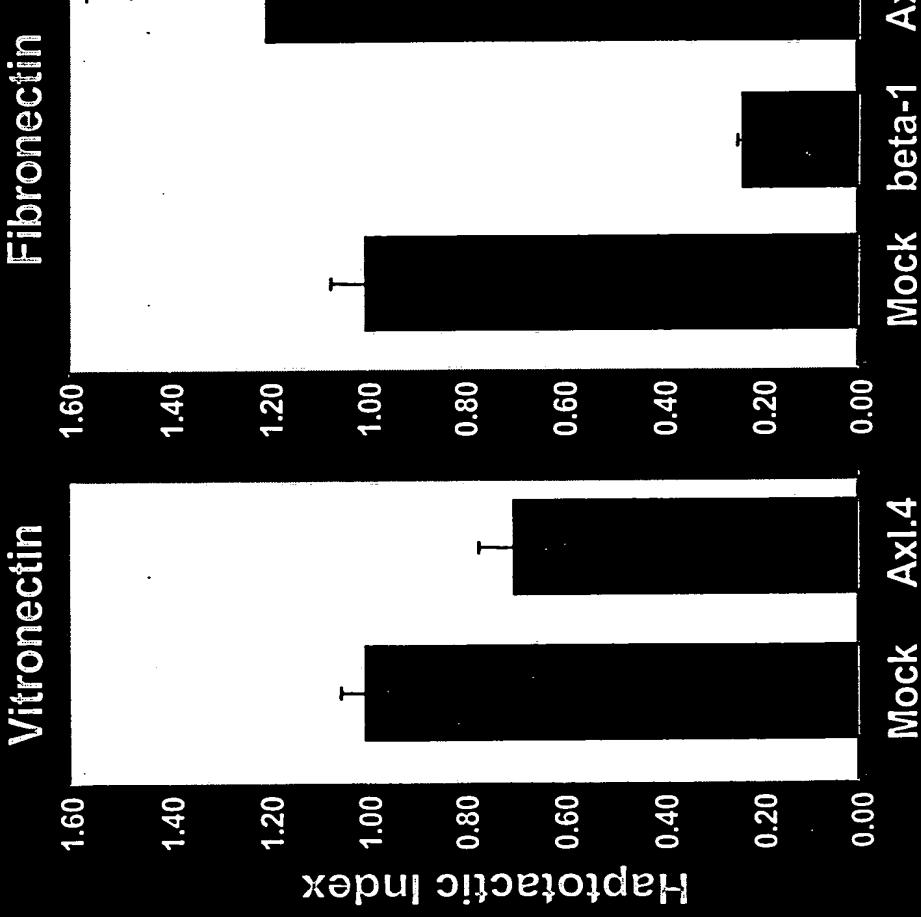


Figure 13

Ax1 RNAi Inhibits Haptotaxis to Vitronectin not Fibronectin

Haptotaxis



Western Blot

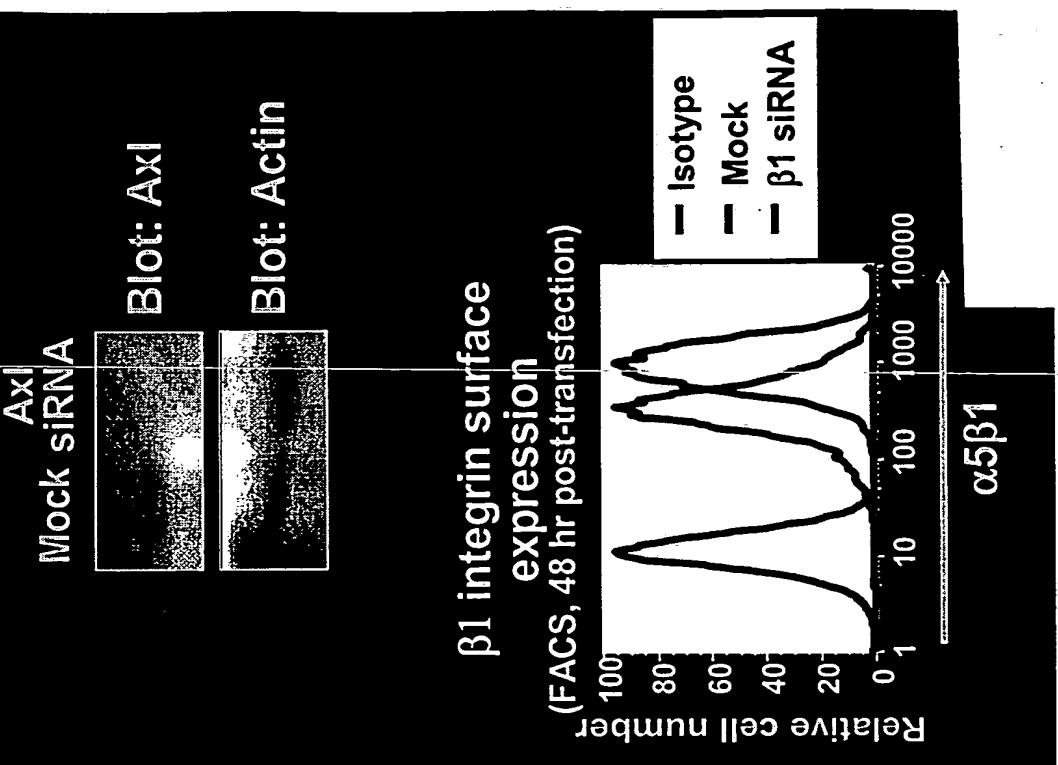


Figure 14

AXR1 RNA Vector Knocks Down AXI Protein Expression in HUVECs

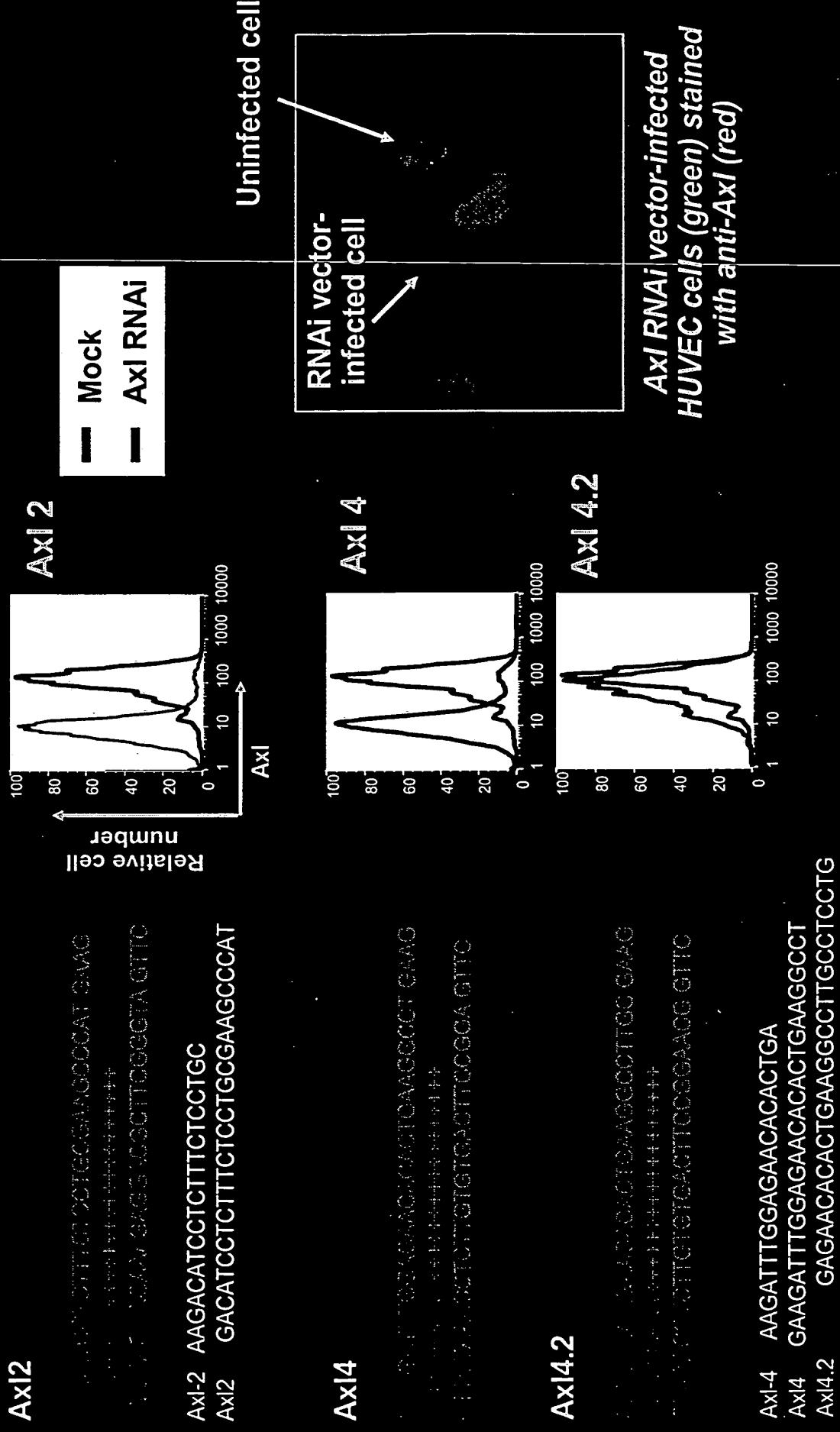


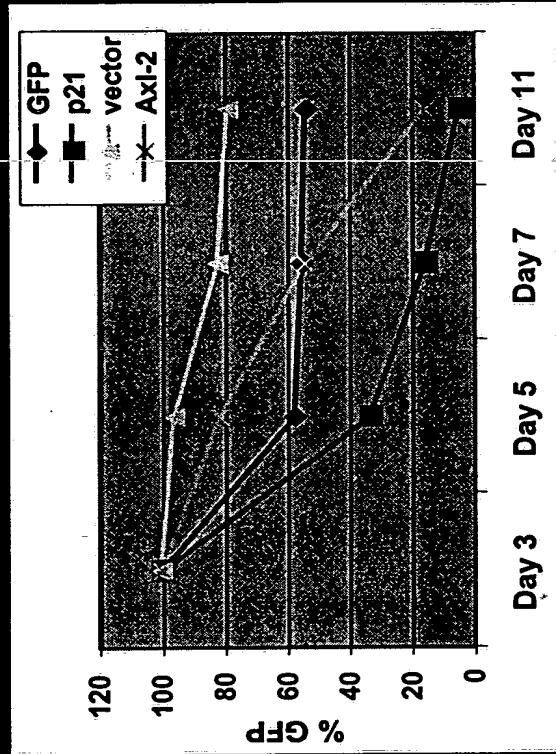
Figure 15

Axl RNAi Vector Inhibits VN Haptotaxis and HUVEC Proliferation

Infect HUVEC with RNAi vector

Follow % GFP over several days

Proliferation (% GFP)



VN Haptotaxis

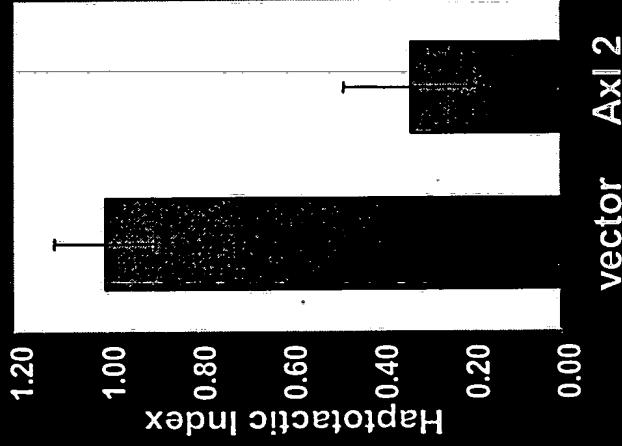


Figure 16

Ax1 Extracellular Domain Was Isolated In VEGFR2 Screen

Clone 921A

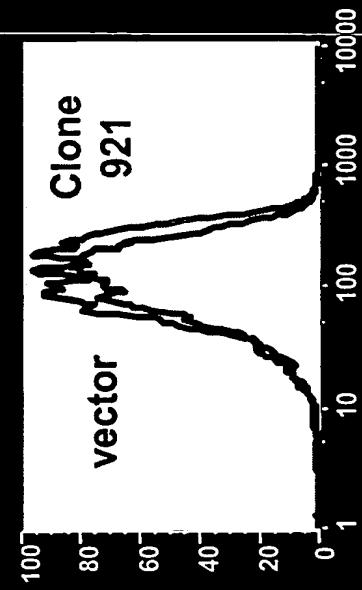
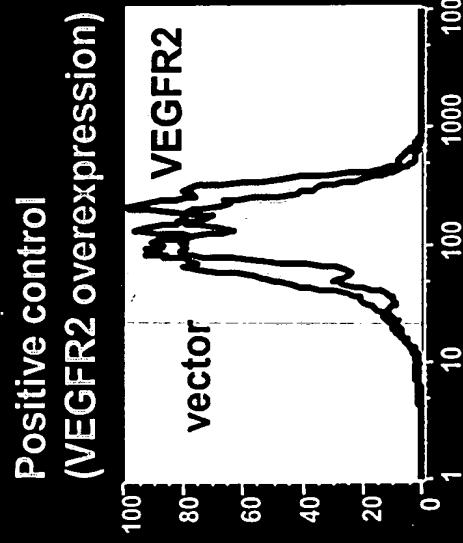


Figure 17

AxI RNAis Inhibit Tube Formation in the Co-Culture Assay

Co-Culture of PASMCS with RNAi-transfected HUVEC Fixed at 5 days; stained with anti-PECAM-FITC

*Western blot; whole cell lysate
48 hrs post transfection*

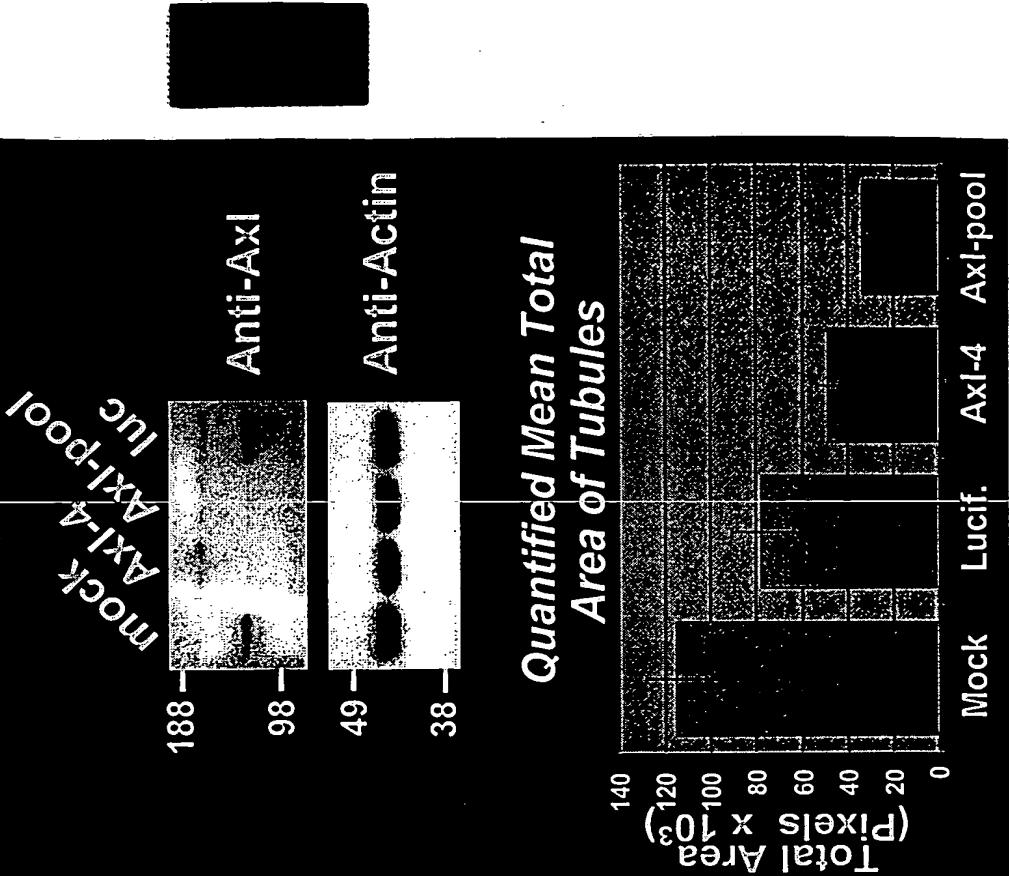
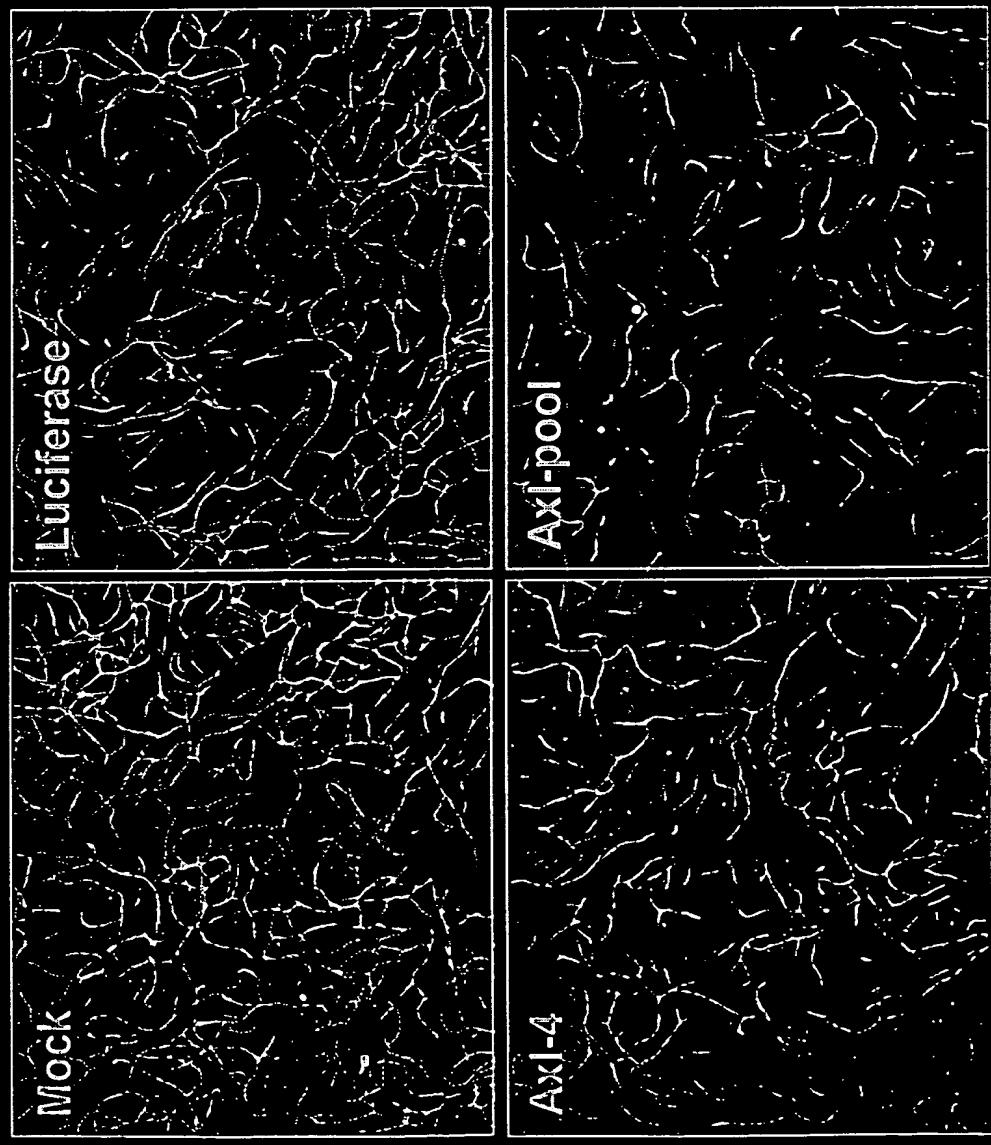


Figure 18

Sponge Angiogenesis / Xenograft Model (Jaques Nör)

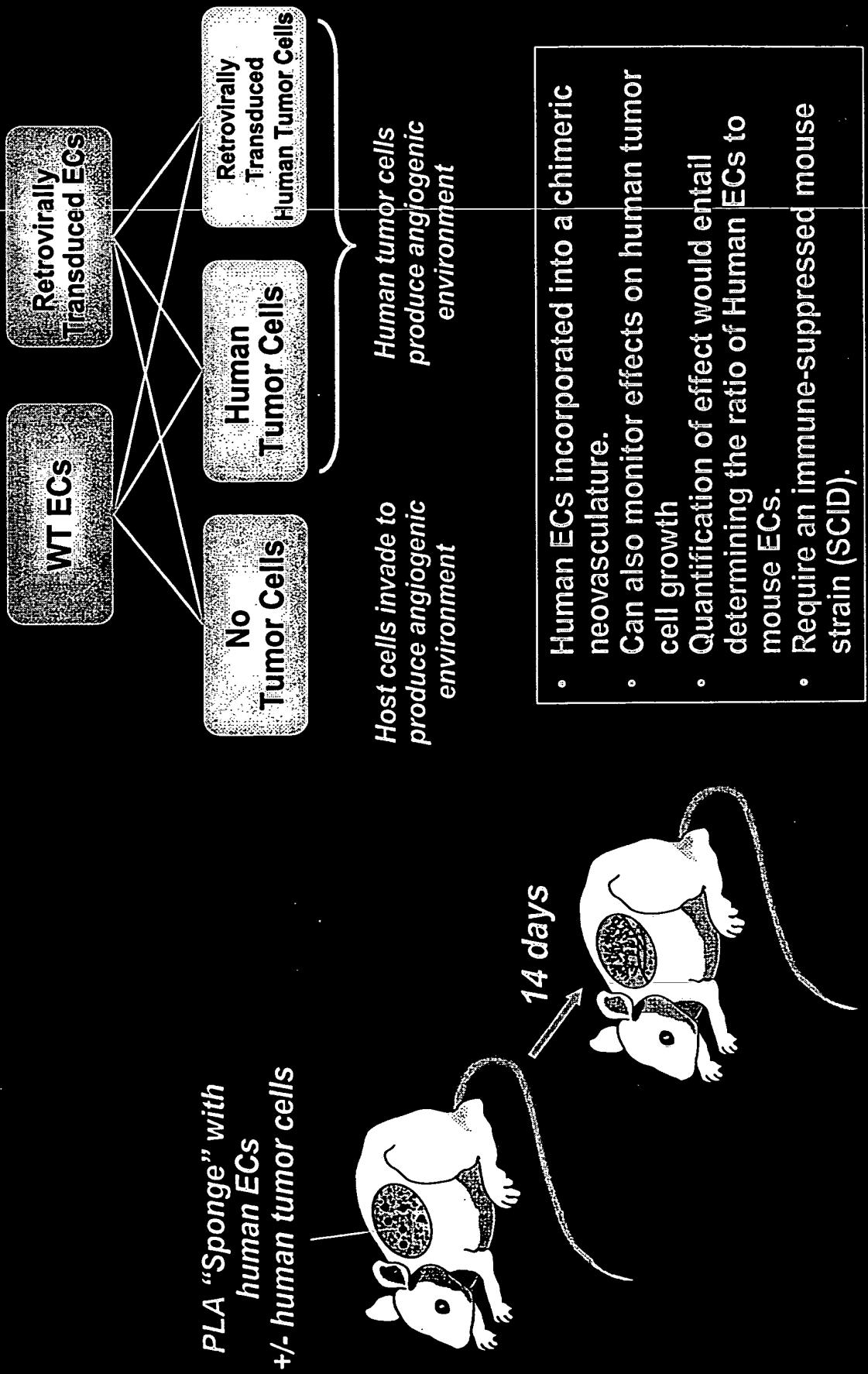
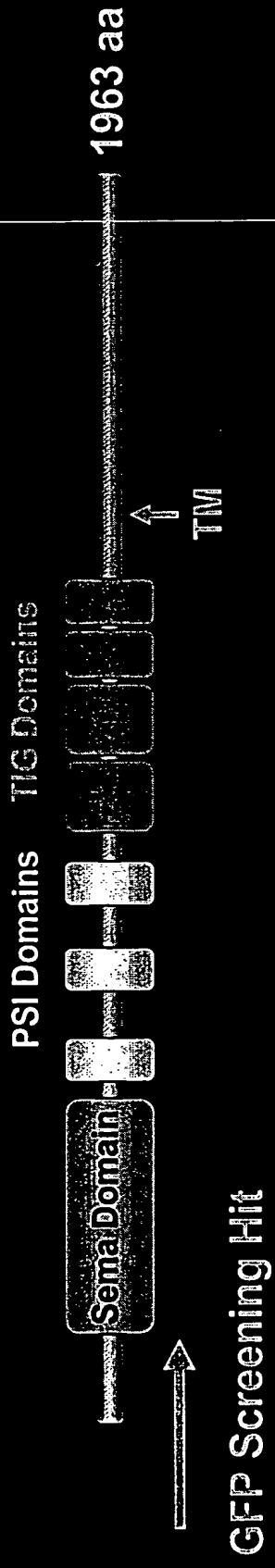


Figure 19

Plexin-A2 (GH1-204)



GFP Screening Hit

Sense hit

- Plexins are semaphorin co-receptors, but lack enzymatic domains
- Plexin A2 co-receptor for Sema3F ($\alpha\beta 3$ screen hit) which has been recently described as anti-angiogenic ligand
- Plexins associate with neuropilins via N-terminus
 - Plexin A2 expression mainly restricted to endothelial cells and brain (SOURCE)

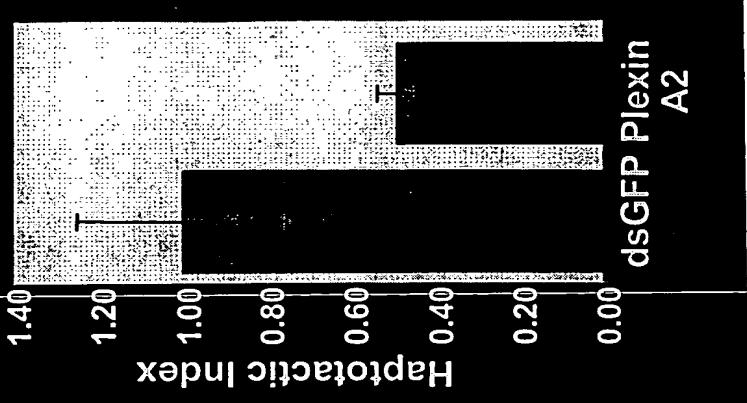
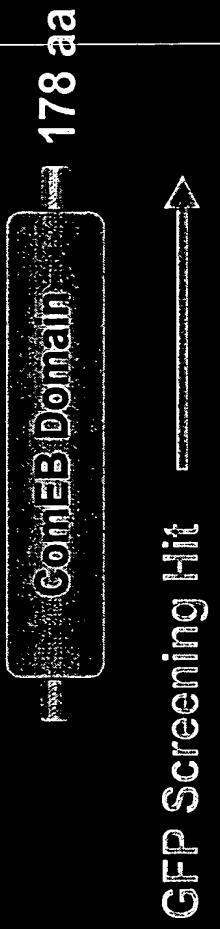


Figure 20

DeOxyCytidine Deaminase (GH1-27)



- Sense hit
- Regulates pyrimidine deoxynucleotide metabolism
- Impairment affects fidelity of DNA replication
- Overexpressed in many malignant tumors and aggressive lymphoid malignancies
- Upregulated following myocardial infarction
- Inhibited by tetrahydrodeoxyuridine (H4U)
- Combination of H4U and 5-fluorodeoxycytidine (2'deoxyctidine kinase substrate) inhibits Lewis Lung carcinoma growth in mouse model

